

## **APPENDIX A: MARKED UP COPY OF CLAIMS**

1. (Amended) A recombinant construct comprising:
  - (a) a DNA sequence encoding a polypeptide having 3-hydroxy-3-methylglutaryl-Coenzyme A reductase enzyme activity, and
  - (b) a DNA sequence encoding a[t least one] polypeptide having [steroid pathway enzyme activity selected from the group consisting of:]
    - squalene epoxidase enzyme activity[,
    - sterol methyl transferase I enzyme activity,
    - sterol C4-demethylase enzyme activity,
    - obtusifoliol D14 $\alpha$ -demethylase enzyme activity,
    - sterol C5-desaturase enzyme activity, and
    - sterol methyl transferase II enzyme activity].
3. (Amended) The recombinant construct of claim 1, further comprising a first promoter operably linked to said DNA sequence encoding a polypeptide having 3-hydroxy-3-methylglutaryl-Coenzyme A reductase enzyme activity and a second promoter operably linked to said DNA sequence encoding squalene epoxidase enzyme activity [at least one polypeptide having steroid pathway enzyme activity], wherein said first and second promoters may or may not be the same.
6. (Amended) A recombinant vector comprising operably linked in the 5' to 3' direction,
  - a promoter, a DNA sequence encoding a polypeptide having a 3-hydroxy-3-methylglutaryl-Coenzyme A reductase enzyme activity, and a transcription termination signal sequence;
  - a promoter, a DNA sequence encoding [at least one polypeptide having steroid pathway enzyme activity selected from the group consisting of]
    - squalene epoxidase enzyme activity,
    - [sterol methyl transferase I enzyme activity,
    - sterol C4-demethylase enzyme activity,
    - obtusifoliol C14  $\alpha$ -demethylase enzyme activity,
    - sterol C5-desaturase enzyme activity, and
    - sterol methyl transferase II enzyme activity,]and a transcription termination signal sequence.
12. (Amended) A transformed host cell comprising a plant expression vector comprising,
  - (a) as operably linked components in the 5' to 3' direction, a promoter, a DNA sequence encoding a polypeptide having a 3-hydroxy-3-methylglutaryl-Coenzyme A reductase enzyme activity, and a transcription termination signal sequence; and

- (b) as operably linked components in the 5' to 3' direction, a promoter, a DNA sequence encoding [at least one polypeptide having steroid pathway enzyme activity selected from the group consisting of]
- squalene epoxidase enzyme activity,
- [sterol methyl transferase I enzyme activity,
- sterol C4-demethylase enzyme activity,
- obtusifoliol C14 $\alpha$ -demethylase enzyme activity,
- sterol C5-desaturase enzyme activity, and
- sterol methyl transferase II enzyme activity,] and a transcription termination signal sequence.
13. The transformed host cell according to claim 12 wherein said host cell is a plant cell.
14. (Amended) A cell culture comprising transformed host cells according to any one of claims 8-13.
15. (Amended) A transformed plant comprising at least one transformed host cell of any one of claims 8-13.
16. A plant according to claim 15 wherein said transformed host cell comprises a plant cell.
17. (Amended) A transformed storage organ, comprising at least one transformed host cell according to any one of claims 8-13.
18. (Amended) A transformed storage organ including at least one transformed host cell containing a recombinant vector comprising:
- (a) As operably linked components in the 5' to 3' direction, a promoter, a DNA sequence encoding at least one polypeptide having 3-hydroxy-3-methylglutaryl-Coenzyme A reductase enzyme activity, and a transcription termination signal sequence; and
- (b) as operably linked components in the 5' to 3' direction, a promoter, a DNA sequence encoding a polypeptide having [steroid pathway enzyme activity selected from the group consisting of]
- squalene epoxidase activity,
- [sterol methyl transferase I enzyme activity,
- sterol C4-demethylase enzyme activity,
- obtusifoliol C14 $\alpha$ -demethylase enzyme activity,
- sterol C5-desaturate enzyme activity, and
- sterol methyl transferase II enzyme activity,] and a transcription termination signal sequence.
19. (Amended) The transformed storage organ according to claim 18 wherein said recombinant vector is a plant expression vector.

20. (Amended) A process of increasing the formation of steroid pathway products in a transformed host cell as compared to an otherwise identical non-transformed host cell comprising:

- (1) transforming a host cell with a recombinant vector comprising
  - (a) as operably linked components in the 5' to 3' direction, a promoter, a DNA sequence encoding a first polypeptide having 3-hydroxy-3-methylglutaryl-Coenzyme A reductase enzyme activity, and a transcription termination signal sequence; and
  - (b) as operably linked components in the 5' to 3' direction, a promoter, a DNA sequence encoding at least one polypeptide having [steroid pathway enzyme activity selected from the group consisting of]
    - squalene epoxidase enzyme activity,
    - [sterol methyl transferase I enzyme activity,
    - sterol C4-demethylase enzyme activity,
    - obtusifoliol C14 $\alpha$ -demethylase enzyme activity,
    - sterol C5-desaturase enzyme activity, and
    - sterol methyl transferase II enzyme activity,]and a transcription termination signal sequence, and

(2) regenerating [said ] a transformed [plant] host cell into said transgenic plant.

25. (Amended) A transgenic plant seed transformed with a vector comprising a DNA segment that encodes a polypeptide having 3-hydroxy-3-methylglutaryl-Coenzyme A reductase activity, and a DNA segment that encodes a polypeptide having [a steroid pathway enzyme activity selected from the group consisting of]

squalene epoxidase enzyme activity,  
[sterol methyl transferase I enzyme activity,  
sterol C4-demethylase enzyme activity,  
obtusifoliol C14 $\alpha$ -demethylase enzyme activity,  
sterol C5-desaturase enzyme activity, and  
sterol methyl transferase II enzyme activity,]

and a promoter suitable for driving expression of said polypeptides in said plant cell, wherein said transgenic plant seed is capable of germinating into a transgenic plant that over-accumulates steroid pathway products relative to a non-transformed plant of the same [strain] species; and mutants, recombinants, genetically engineered derivatives thereof and hybrids derived therefrom, wherein said mutants, recombinants, genetically engineered derivatives thereof and hybrids derived therefrom maintain the ability to overaccumulate steroid pathway products.

26. (Amended) A plant, the genome of which includes introduced DNA comprising:

DNA encoding a polypeptide having 3-hydroxy-3-methylglutaryl-Coenzyme A reductase enzyme activity, wherein said plant contains an elevated level of total accumulated sterol, compared to an otherwise identical plant, the genome of which does not comprise said introduced DNA encoding a polypeptide encoding 3-hydroxy-3-methylglutaryl-Coenzyme A reductase enzyme activity; and

further comprising introduced DNA encoding at least one polypeptide having [steroid pathway enzyme activity selected from the group consisting of]

squalene epoxidase enzyme activity,  
[sterol methyl transferase I enzyme activity,  
sterol C4-demethylase enzyme activity,  
obtusifolioside C14 $\alpha$ -demethylase enzyme activity,  
sterol C5-desaturase enzyme activity, and  
sterol methyl transferase II enzyme activity,]

wherein said introduced DNAs are operatively linked to regulatory signals that cause seed-specific expression of said introduced DNAs, and wherein seeds of said plant contain a reduced level of squalene, cycloartenol, 24-methylene cycloartenol, obtusifolioside, stigmasterol-7-enol, or campesterol compared to the seeds of an otherwise identical plant whose genome does not contain introduced DNA encoding said at least one polypeptide having [steroid pathway enzyme] squalene epoxidase enzyme activity.

27. (Amended) A plant, the genome of which includes an introduced DNA sequence encoding a polypeptide having 3-hydroxy-3-methylglutaryl-Coenzyme A reductase activity and an introduced DNA sequence encoding at least one polypeptide having squalene epoxidase enzyme activity, [sterol methyl transferase I enzyme activity, sterol C4-demethylase enzyme activity, obtusifolioside C14 $\alpha$ -demethylase enzyme activity, sterol C5-desaturase enzyme activity, or sterol methyl transferase II enzyme activity,] wherein said introduced DNA is operably linked to regulatory signals that cause seed-specific expression of said introduced DNA, and wherein said plant produces seed having an elevated level of a steroid pathway product, compared to a corresponding transgenic or non-transgenic plant that does not contain said introduced DNA.

28. (Amended) A plant comprising introduced DNA encoding (i) a polypeptide having 3-hydroxy-3-methylglutaryl-Coenzyme A reductase enzyme activity and (ii) at least one additional polypeptide having squalene epoxidase enzyme activity, [sterol methyl transferase I enzyme activity, sterol C4-demethylase enzyme activity, obtusifolioside C14 $\alpha$ -demethylase enzyme activity, sterol C5-desaturase enzyme activity, or sterol methyl transferase II enzyme activity,] wherein said plant that produces a storage organ having an elevated level of a sterol pathway product compared to a corresponding transgenic or non-transgenic plant that does not contain said introduced DNA.

29. (Amended) The plant of claim 28, wherein said storage organ contains a reduced level of squalene, cycloartenol, 24-methyl cycloartenol, obtusifolioside, stigmasterol-7-enol, campesterol, or mixtures thereof, compared to a corresponding transgenic plant that comprises introduced DNA encoding a polypeptide having 3-hydroxy-3-methylglutaryl-Coenzyme A reductase enzyme activity but that does not contain introduced DNA encoding at least one polypeptide having squalene epoxidase enzyme activity[, sterol methyl transferase I enzyme activity, sterol C4-

demethylase enzyme activity, obtusifolioside C14 $\alpha$ -demethylase enzyme activity, sterol C5-desaturase enzyme activity, sterol methyl transferase II enzyme activity].

31. (Amended) A transformed seed of a plant according to any one of claims 24 to 30.
32. (Amended) Transgenic [P]progeny of a plant according to any one of claims 24 to 30.
33. (Amended) A transformed plant cell or transformed plant cell of a plant according to any one of claims 24 to 30.
35. (Amended) A transformed plant produced from a seed according to claim 31.
67. (Amended) The transformed storage organ of claim 18, wherein said at least one transformed host cell further contains a recombinant vector comprising as operably linked components, a promoter, a DNA sequence encoding a tocopherol synthesis pathway enzyme and a transcription termination sequence.
68. (Amended) The transformed storage organ of claim 18, wherein said tocopherol synthesis enzyme is S-adenosylmethionine-dependent  $\gamma$ -tocopherol methyltransferase.

## **APPENDIX B: CLEAN COPY OF CLAIMS**

1. (Amended) A recombinant construct comprising:
  - (a) a DNA sequence encoding a polypeptide having 3-hydroxy-3-methylglutaryl-Coenzyme A reductase enzyme activity, and
  - (b) a DNA sequence encoding a polypeptide having squalene epoxidase enzyme activity.
2. The recombinant construct of claim 1, further comprising at least one promoter operably linked to said coding regions.
3. (Amended) The recombinant construct of claim 1, further comprising a first promoter operably linked to said DNA sequence encoding a polypeptide having 3-hydroxy-3-methylglutaryl-Coenzyme A reductase enzyme activity and a second promoter operably linked to said DNA sequence encoding squalene epoxidase enzyme activity, wherein said first and second promoters may or may not be the same.
4. The recombinant construct of claim 2 or 3 further comprising an operably linked transcription termination sequence located 3' to each coding region.
5. A recombinant construct according to claim 3 wherein the promoters are selected from the group consisting of seed-specific promoters, organ specific promoters and constitutive promoters.
6. (Amended) A recombinant vector comprising operably linked in the 5' to 3' direction,
  - a promoter, a DNA sequence encoding a polypeptide having a 3-hydroxy-3-methylglutaryl-Coenzyme A reductase enzyme activity, and a transcription termination signal sequence;
  - a promoter, a DNA sequence encoding squalene epoxidase enzyme activity, and a transcription termination signal sequence.
7. The recombinant vector of claim 6 wherein said vector is a plant expression vector.
8. A transformed host cell comprising a recombinant construct of claim 1.
9. The transformed host cell of claim 8 wherein said cell is a plant cell.
10. A transformed host cell comprising a recombinant vector of claim 6.
11. The transformed host cell according to claim 10 wherein said host cell is a plant cell.
12. (Amended) A transformed host cell comprising a plant expression vector comprising,
  - (a) as operably linked components in the 5' to 3' direction, a promoter, a DNA sequence encoding a polypeptide having a 3-hydroxy-3-methylglutaryl-Coenzyme A reductase enzyme activity, and a transcription termination signal sequence; and
  - (b) as operably linked components in the 5' to 3' direction, a promoter, a DNA sequence encoding squalene epoxidase enzyme activity, and a transcription termination signal sequence.
13. The transformed host cell according to claim 12 wherein said host cell is a plant cell.

14. (Amended) A cell culture comprising transformed host cells according to any one of claims 8-13.
15. (Amended) A transformed plant comprising at least one transformed host cell of any one of claims 8-13.
16. A plant according to claim 15 wherein said transformed host cell comprises a plant cell.
17. (Amended) A transformed storage organ, comprising at least one transformed host cell according to any one of claims 8-13.
18. (Amended) A transformed storage organ including at least one transformed host cell containing a recombinant vector comprising:
  - (a) As operably linked components in the 5' to 3' direction, a promoter, a DNA sequence encoding at least one polypeptide having 3-hydroxy-3-methylglutaryl-Coenzyme A reductase enzyme activity, and a transcription termination signal sequence; and
  - (b) as operably linked components in the 5' to 3' direction, a promoter, a DNA sequence encoding a polypeptide having squalene epoxidase activity, and a transcription termination signal sequence.
19. (Amended) The transformed storage organ according to claim 18 wherein said recombinant vector is a plant expression vector.
20. (Amended) A process of increasing the formation of steroid pathway products in a transformed host cell as compared to an otherwise identical non-transformed host cell comprising:
  - (1) transforming a host cell with a recombinant vector comprising
    - (a) as operably linked components in the 5' to 3' direction, a promoter, a DNA sequence encoding a first polypeptide having 3-hydroxy-3-methylglutaryl-Coenzyme A reductase enzyme activity, and a transcription termination signal sequence; and
    - (b) as operably linked components in the 5' to 3' direction, a promoter, a DNA sequence encoding at least one polypeptide having squalene epoxidase enzyme activity, and a transcription termination signal sequence, and
  - (2) regenerating a transformed host cell into said transgenic plant.
21. The process according to claim 20 wherein said first encoded polypeptide comprises the catalytic region and at least a portion of the linker region but is free from the membrane binding region of a 3-hydroxy-3-methylglutaryl-Coenzyme A reductase enzyme.
22. The process according to claim 20 wherein said promoters are promoters whose regulatory function is substantially unaffected by levels of squalene in said transgenic plant.
23. The process according to claim 20 wherein said plant cell is selected from the group consisting of canola, soybean, corn, tobacco, cotton, tomato, potato, safflower, sunflower, peanut, rape, flax, oil, palm, cuphea and alfalfa.
24. A transgenic plant produced in accordance with the process of claim 20.

25. (Amended) A transgenic plant seed transformed with a vector comprising a DNA segment that encodes a polypeptide having 3-hydroxy-3-methylglutaryl-Coenzyme A reductase activity, and a DNA segment that encodes a polypeptide having squalene epoxidase enzyme activity, and a promoter suitable for driving expression of said polypeptides in said plant cell, wherein said transgenic plant seed is capable of germinating into a transgenic plant that overaccumulates steroid pathway products relative to a non-transformed plant of the same species; and mutants, recombinants, genetically engineered derivatives thereof and hybrids derived therefrom, wherein said mutants, recombinants, genetically engineered derivatives thereof and hybrids derived therefrom maintain the ability to overaccumulate steroid pathway products.

26. (Amended) A plant, the genome of which includes introduced DNA comprising:

DNA encoding a polypeptide having 3-hydroxy-3-methylglutaryl-Coenzyme A reductase enzyme activity, wherein said plant contains an elevated level of total accumulated sterol, compared to an otherwise identical plant, the genome of which does not comprise said introduced DNA encoding a polypeptide encoding 3-hydroxy-3-methylglutaryl-Coenzyme A reductase enzyme activity; and

further comprising introduced DNA encoding at least one polypeptide having squalene epoxidase enzyme activity,

wherein said introduced DNAs are operatively linked to regulatory signals that cause seed-specific expression of said introduced DNAs, and wherein seeds of said plant contain a reduced level of squalene, cycloartenol, 24-methylene cycloartenol, obtusifoliol, stigmasta-7-enol, or campestral compared to the seeds of an otherwise identical plant whose genome does not contain introduced DNA encoding said at least one polypeptide having squalene epoxidase enzyme activity.

27. (Amended) A plant, the genome of which includes an introduced DNA sequence encoding a polypeptide having 3-hydroxy-3-methylglutaryl-Coenzyme A reductase activity and an introduced DNA sequence encoding at least one polypeptide having squalene epoxidase enzyme activity, wherein said introduced DNA is operably linked to regulatory signals that cause seed-specific expression of said introduced DNA, and wherein said plant produces seed having an elevated level of a steroid pathway product, compared to a corresponding transgenic or non-transgenic plant that does not contain said introduced DNA.

28. (Amended) A plant comprising introduced DNA encoding (i) a polypeptide having 3-hydroxy-3-methylglutaryl-Coenzyme A reductase enzyme activity and (ii) at least one additional polypeptide having squalene epoxidase enzyme activity, wherein said plant that produces a storage organ having an elevated level of a sterol pathway product compared to a corresponding transgenic or non-transgenic plant that does not contain said introduced DNA.

29. (Amended) The plant of claim 28, wherein said storage organ contains a reduced level of squalene, cycloartenol, 24-methyl cycloartenol, obtusifoliol, stigmasta-7-enol, campesterol, or mixtures thereof, compared to a corresponding transgenic plant that comprises introduced DNA encoding a polypeptide having 3-hydroxy-3-methylglutaryl-Coenzyme A reductase enzyme activity but that does not contain introduced DNA encoding at least one polypeptide having squalene epoxidase enzyme activity.

30. The plant of any one of claims 24 to 29, wherein said regulatory signals cause seed-specific expression of said introduced DNAs.



31. (Amended) A transformed seed of a plant according to any one of claims 24 to 30.
32. (Amended) Transgenic progeny of a plant according to any one of claims 24 to 30.
33. (Amended) A transformed plant cell or transformed plant cell of a plant according to any one of claims 24 to 30.
35. (Amended) A transformed plant produced from a seed according to claim 31.
63. The transformed host cell of claim 8, further comprising a recombinant construct encoding a tocopherol synthesis pathway enzyme.
64. The transformed host cell of claim 10, further comprising a recombinant vector encoding a tocopherol synthesis pathway enzyme.
65. The transformed host cell of claim 64, wherein said tocopherol synthesis pathway enzyme is S-adenosylmethionine-dependent  $\gamma$ -tocopherol methyltransferase.
66. The transformed host cell of claim 64, wherein said vectors are plant expression vectors.
67. (Amended) The transformed storage organ of claim 18, wherein said at least one transformed host cell further contains a recombinant vector comprising as operably linked components, a promoter, a DNA sequence encoding a tocopherol synthesis pathway enzyme and a transcription termination sequence.
68. (Amended) The transformed storage organ of claim 18, wherein said tocopherol synthesis enzyme is S-adenosylmethionine-dependent  $\gamma$ -tocopherol methyltransferase.
34. A cell culture, comprising cells according to claim 33.
38. A plant according to any one of claims 24 to 30, wherein said plant is an apomictic plant.
40. A uniform population of plants according to any one of claims 15, 16, 24-30, 37 or 38.
42. A part, other than a seed, of a plant according to any one of claims 15, 16, 24-30, 37 or 38.